

# Breeding Improved Varieties of White Clover



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## Summary

White clover is one of the most nutritious species available in grassland/ruminant production systems. In association with grass, this species increases protein, mineral content, intake and nutrient value of the total forage. Because of its nitrogen fixing capacity, white clover has the potential to reduce, or in the case of organic systems, eliminate the need for inorganic nitrogen fertilizer on grazed grassland.

Grassland-based animal production is a major part of the Irish agricultural economy, consequently any improvement in this legume has large potential benefit in this sector.

The background, methodology, objectives and output of the current Teagasc, Oak Park white clover breeding programme are outlined.

Five varieties have been released and are currently on Recommended Lists in Ireland and elsewhere. Aran, first released in 1981, has remained the highest yielding clover variety in UK trials, it is also widely grown in New Zealand, Australia and France as well as Ireland and UK. Avoca has shown very good yield and persistency under a range of managements and is widely used in Ireland and UK. Chieftain, the most recently released variety, has given 25% more clover yield than the control under lax defoliation (simulated grazing) management in UK Recommended List trials.

In parallel with the breeding programme, research on nitrogen fixation and development of inbred lines in this species was undertaken. A brief summary of some of the results is included in this report.

## Introduction

Approximately 80% of milk and 60% of beef production comes from grazed grass/clover pastures. Genetic improvement of component species through breeding of better varieties (especially white clover) increases the potential productivity of our grasslands. White clover is a key component of good, productive pasture.

Varieties which are persistent, high yielding and adapted to a range of management systems are required to underpin the exploitation of good grass/clover production systems. Benefits include: increased biological nitrogen fixation, increased quality/intake of pasture, reduced dependence on inorganic N<sub>2</sub> fertilizer and reduced costs.

White clover is an allogamous allotetraploid species and productivity is measured in association with a companion grass with multiple harvests per year. This makes

measurement and identification of superior genotypes/families difficult. Despite these problems of measurement, substantial improvement in varietal performance has been achieved (Woodfield and Caradus, 1994, Rhodes and Ortega, 1997).

## Materials and methods

### Genetic variation

In a breeding programme, it is very important to ensure that response to selection is not restricted by lack of genetic variation. Germplasm from three sources has been used in the programme.

1. Existing bred varieties which are widely grown in Europe and New Zealand.
2. Introductions from gene banks, especially from USDA sources. These represent collections made in various climatic regions and contain desirable variation for specific traits such as out of season growth, disease resistance, etc.
3. Collections of germplasm in Ireland from old pastures which had no prior history of reseeded. An extensive collection from old pastures was made in the period 1979-1983. This was supported by the EC; some seed from each collection site was distributed to each EC country. Residue seed is maintained in long-term storage at Oak Park. Details of this collection, multiplication and storage were published (Connolly *et al.*, 1989; Connolly, 2000).

### Breeding system and methodology

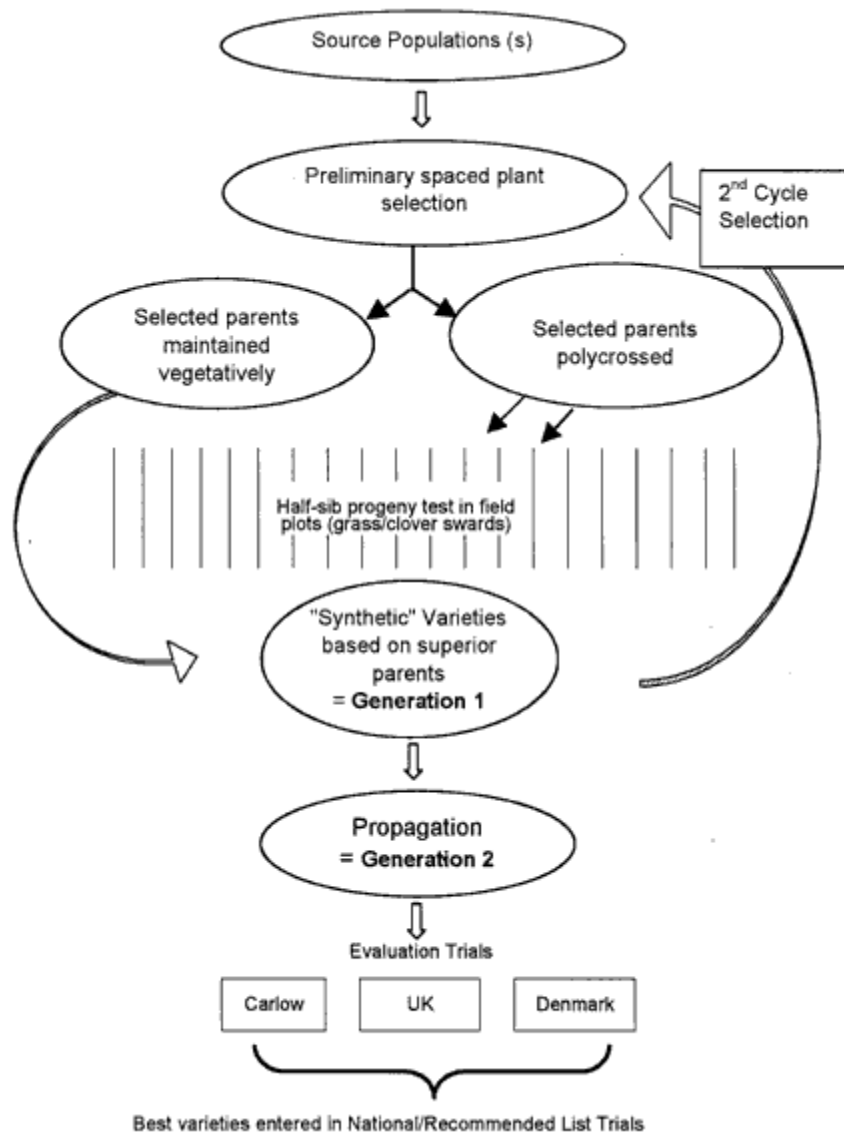
White clover is a cross-pollinating species, this determines, to a large extent, the selection strategy used. In addition, individual genotype performance (measured as spaced plants) does not predict field results under competitive sward conditions for many characters, such as yield and persistency.

This problem of evaluation is further complicated by the fact that white clover is always grown in association with grass. Consequently the selection procedure is based largely on full-sib or half-sib progeny tests in mixed grass/clover swards. The system is designed to bring the families under selection to field plot stage as early as possible in the selection programme.

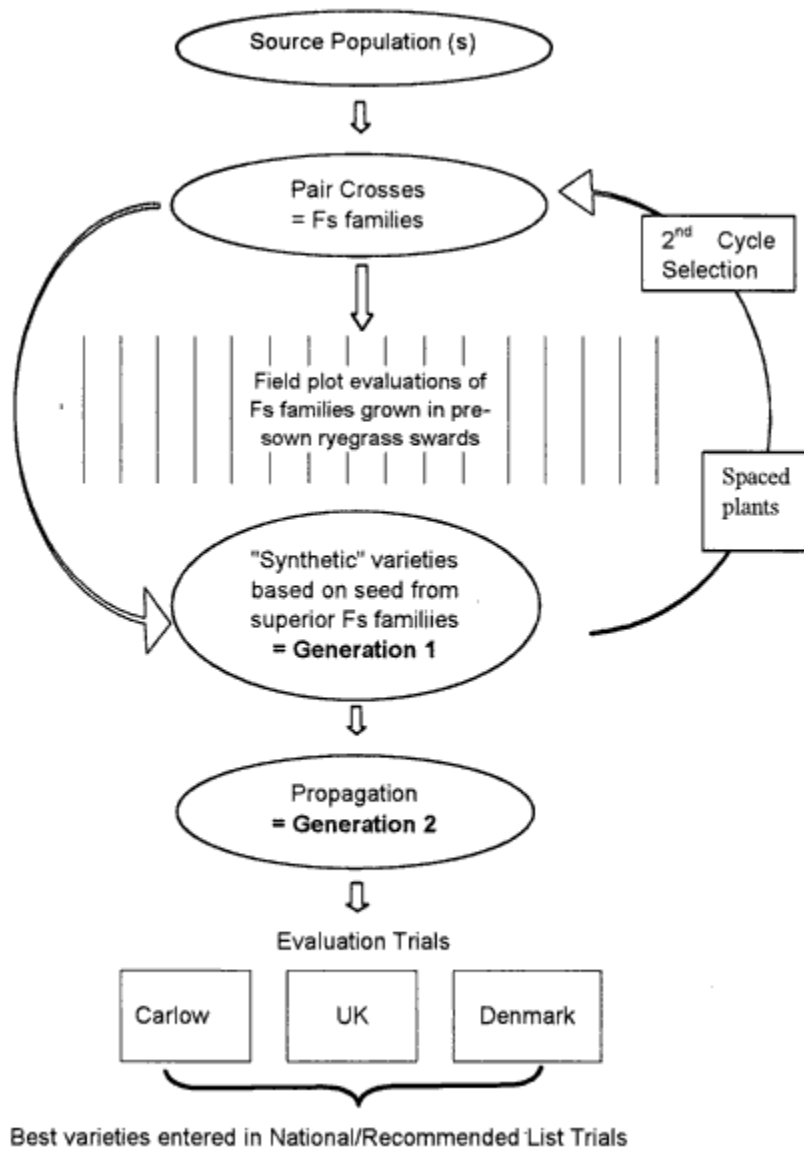
Half-sib seed is derived from polycrosses in which the parent genotypes have been clonally propagated so that adequate seed is obtained from each genotype for field trials. Preliminary spaced-plant selection is necessary to ensure that parents included in each polycross have similar dates of flowering. Randomisation of clonal propagations plus similarity in heading date is required to ensure random pollination of the polycross group. Because white clover is pollinated by bees each polycross is isolated in a modified polythene tunnel. Nucleus beehives are introduced at flowering for pollination (Fig. 1). The half-sib progeny test procedure is summarised in Fig. 2.



**Fig. 1:** Bee proof pollination "houses" for production of half-sib polycross families - note nucleus beehive in centre (right)



**Fig. 2:** Half-sib progeny test selection : White Clover



**Fig. 3:** Full-sib family selection : White Clover

An alternative breeding system based on recurrent full-sib (FS) family selection is also used. FS families are produced by pair crossing selected parents. These crosses are produced by hand pollination. Fig. 3 illustrates one cycle of selection for the full-sib (FS) family procedure.

Half-sib and full-sib families are established (in combination with ryegrass) in field plots for progeny test trials. Two contrasting managements are imposed on the replicated family plots - persistence management (simulated intensive grazing, 12-14 cuts/year) and yield management (6-8 cuts per year). Field evaluation requires a minimum of 3 years. One complete cycle of selection, from initial pair crossing to final test results, requires 5 years.



**Fig. 4:** Illustrates variation for vigour/persistency between swards of full-sib families (intensive grazing left, yield management right)

New synthetic varieties are based on combinations of superior families. These new varieties are evaluated at Oak Park and DLF test centres in UK, Denmark and Germany. The best synthetics are multiplied and submitted to National/Recommend List evaluations systems in Ireland, UK and other countries where appropriate. Only varieties, which are accepted on to Recommended Lists, are propagated and marketed.

## Objectives

- Increased persistency, especially in the medium and large leaf size categories.
- Increased yield, both seasonal and annual.
- Improved stolon density (medium and large leaf types).
- Improved disease/pest resistance.
- Good seed production potential.

## Results

### Varieties released

A list of the varieties released and the countries in which they are included in the Recommended List is given in Table 1.

Table 1: Varieties released

Name	Type	Recommended Listing
Aran	Large	Ireland, N. Ireland, England, Scotland, France, New Zealand, Victoria (Australia)
Avoca	Medium	Ireland, N. Ireland, England, Scotland
Susi	Medium	Ireland, France
Tara	Small	Ireland, N. Ireland, Scotland
Chieftain	Large	England, Scotland, N. Ireland

Table 2 summarises data from various countries for Aran and Avoca.

**Table 2:** Summary of yield data for Aran and Avoca clover varieties.

Data Source: Recommended List trials in various countries.

Results expressed as % of designated control variety and averaged over sites.

Country	Control variety (=100)	No of sites	Variety	Yield	Note
Ireland	Susi	4	<b>Aran</b>	108	Grass + clover yield
N. Ireland	Huia	1		137	Yield of clover component
England	Menna	5		140	Yield of clover component
Scotland	Alice	3		99	Yield of clover component
N. Zealand <sup>1</sup>	Huia	1		125	Grass + clover yield
N. Zealand <sup>2</sup>	Huia	1		123	Grass + clover yield
Victoria (Aus) <sup>3</sup>	Kopu	4		105	Yield of clover component
Ireland	Susi	4	<b>Avoca</b>	104	Grass + clover yield
N. Ireland	Huia	1		110	Yield of clover component
England	Menna	5		107	Yield of clover component
Scotland	Huia	3		157	Yield of clover component

<sup>1,2</sup> Simulated on farm conditions: Rotational grazing with dairy cows, rotational grazing with sheep, respectively

<sup>3</sup> Rotational grazing with cattle and/or sheep

Aran is a very vigorous large-leaved type which competes well with companion grasses, shows good tolerance to applied nitrogen and gives high output under good rotational management.

Avoca is a medium-leaved type with good yield and persistency under a range of management systems.

## Commercialisation

Prior to 1992 various seed companies marketed white clover varieties bred at Oak Park as follows:

- Germinal Holding, Banbridge, Co. Down.
- Goldcrop Ltd., Cork.

An agreement with DLF-Trifolium, Denmark regarding financial support for the breeding programme was finalised in 1992.

In return for financial support DLF-Trifolium have world-wide propagation and marketing rights on all new varieties which emerge from the programme. DLF-Trifolium is one of the largest seed companies in the world. They have breeding and research programmes based in Denmark, France, Norway and Czech Republic with trial facilities in most EU countries and USA. This agreement gives Teagasc access to a wide variety evaluation network and also the opportunity to link into the basic research work in progress at DLF-Trifolium laboratories.

## Research

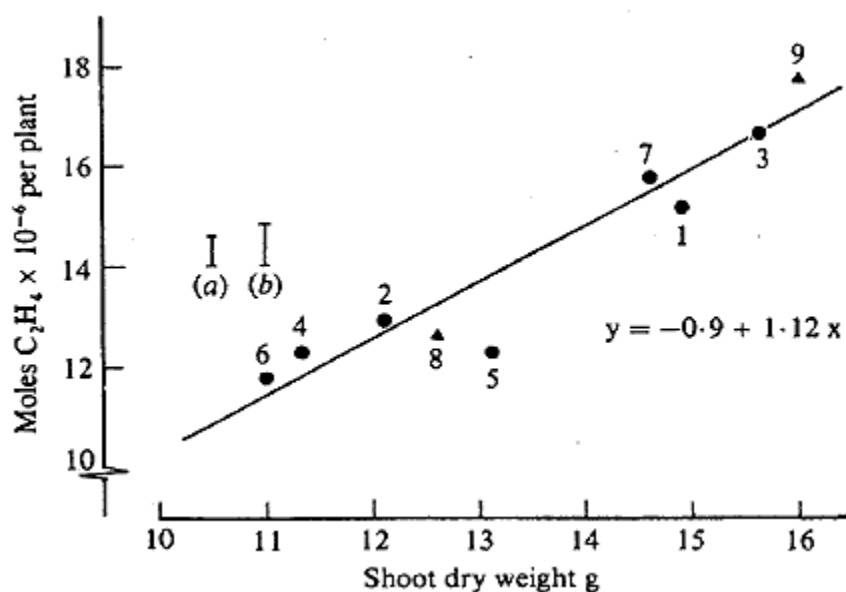
Research on a number of topics closely related to the objectives of the breeding programme was undertaken in parallel with the selection work.

### Nitrogen fixation

N<sub>2</sub> fixation is a primary function of white clover in the pasture, consequently, information on the genetic control of this character is important. Genetic analysis of nodulation and N<sub>2</sub> fixation under laboratory and field conditions were undertaken. The results showed that there was genetic variation for various components of the fixation process, such as time of nodulation, nodule number, nodule volume and plant vigour (Connolly *et al*, 1969). However, there was no correlation between laboratory and field results and this approach to selection for improved fixation rate was terminated.

At a later stage a new method of estimating N<sub>2</sub> fixation based on the acetylene/ethylene reduction assay procedure for measuring nitrogenase activity was developed (Stewart *et al*, 1967; Hardy *et al*, 1968). This technique was modified and applied to grass/clover swards *in situ* under field conditions (Connolly and O'Keeffe, 1979). It was concluded that much of the variation on nitrogenase activity was associated with clover mass.

Selection for increased clover yield and competitive ability was considered the most effective means of increasing nitrogen fixation under practical field conditions.



**Fig. 5:** Relationship between ethylene output per plant and shoot dry weight for harvest 2. Hybrid populations (a) s.e. for population means 1,2,5,6 and 7. (b) s.e. for population means 3,4, 8 and 9.

## Inbreeding/self-incompatibility

Development and selection of inbred lines and their use in the production of synthetic varieties provides an alternative breeding procedure for this species. In general inbreeding depression is severe in white clover (Fig. 6). However, there is variability in the degree of depression, and selection for improved vigour can be achieved. Inbred lines are very useful for genetic analysis, in addition, they can be characterised, evaluated and recombined to form synthetics based on general combining ability analysis. If such lines can be generated in large numbers then they offer an alternative means of developing improved varieties. White clover has a very strong self-incompatibility system, consequently, production of inbred lines by self-pollination is difficult. Research on the use of high temperatures and *in vitro* pollination to overcome/suppress the self-incompatibility mechanism was undertaken (Douglas and Connolly, 1989; Leduc *et al*, 1990). In general, temperature treatment of plants was ineffective in increasing seed set on self-pollination. Some results are summarised in Table 3.



**Table 3:** Effect of elevated temperature (35°C) for 1 day post self-pollination on seed set in *T. repens*.

Data averaged over 3 seasons

Temperature	No. genotypes tested	No. florets selfed	Seed per 100 florets
35°C	60	34248	4.1
Ambient	68	55114	3.4
For comparative purposes seed set following manual cross pollination averaged over 3 seasons was 206 per 100 florets (Douglas & Connolly)			



**Fig. 6:** Inbreeding depression in white clover (non-inbred on left, inbred on right)

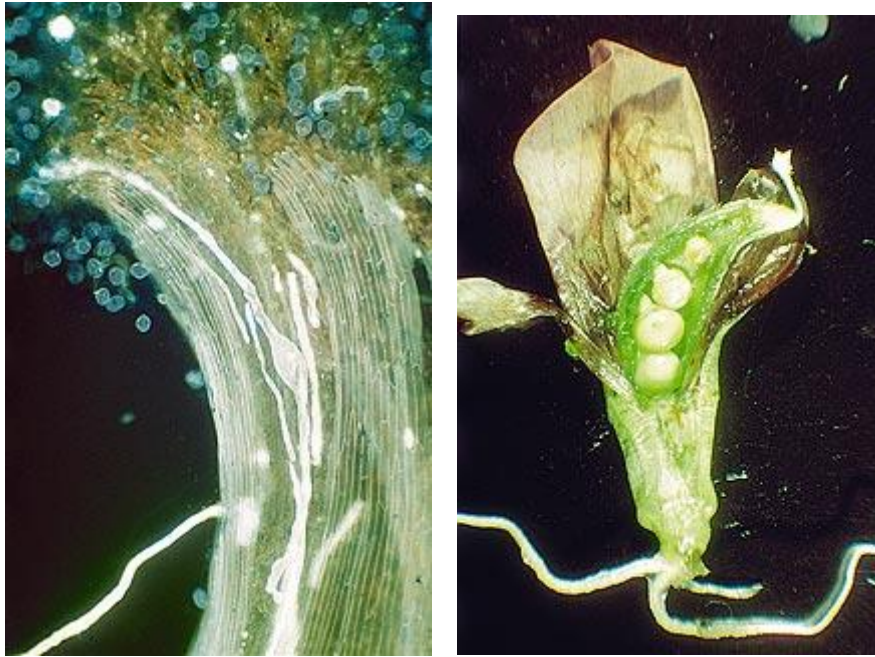
Culture and self pollination of florets *in vitro* gave, on average, a 4-fold increase in seed set per 100 florets (Fig. 7). Comparative results for *in situ* and *in vitro* self pollination for genotypes with varying levels of self-incompatibility are given in Table 4. These results indicate that *in vitro* techniques can be used to suppress self-incompatibility even for genotypes which have a high level of expression of this out-breeding system. Some genotypes, (e.g. genotype J in Table 4), with a high level of self-compatibility were also found as part of this programme.

**Table 4:** Seed set following self-pollination *in situ* and *in vitro* on a range of genotypes in *T. repens*

Category*	Genotype	Seeds/100 florets			
		<i>In situ</i>		<i>In vitro</i>	
S	A	0.31	(319)**	8.5	(35)
	B	0.30	(328)	3.1	(65)
	C	0.63	(315)	3.4	(29)
	D	0.34	(293)	10.8	(37)
	E	0.69	(722)	7.4	(54)
M	F	1.58	(569)	28.6	(69)
	G	9.00	(332)	66.6	(18)
	H	2.00	(759)	0.0	(34)
	I	3.30	(695)	32.0	(25)
W	J	111.0	(191)	186.3	(22)
	<b>Mean</b>	<b>6.6</b>		<b>24.5</b>	

\*Category S, M, W = genotypes with strong, medium and weak self- incompatibility based on *in situ* responses, respectively.

\*\*No of florets selfed in parenthesis.



**Fig. 7:** Left: Germination and growth of pollen tubes in style

Right: Section through seed pod showing seed development following self pollination *in vitro*

## Conclusions

- Five varieties of white clover have been released and are currently on various Recommended Lists.
- New varieties emerging from the recurrent full-sib family selection are currently in trial.
- Development of inbred lines offers new possibilities in variety production.

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